Preconditioning with lipopolysaccharide activates spinal cord microglia without causing neuropathology

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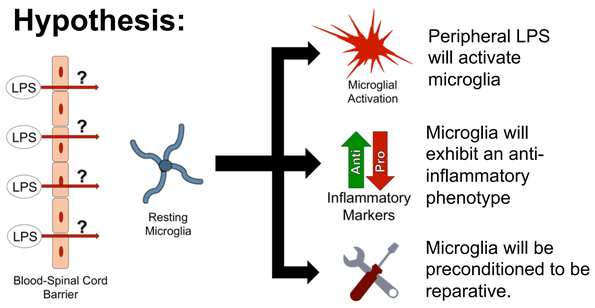
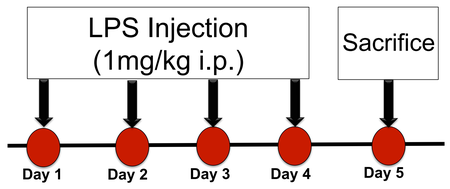
Introduction

Neuroinflammation results from a cellular cascade largely orchestrated by resident and infiltrating immune cells. Microglia, the resident immune cells of the central nervous system (CNS), function as sentinels in the normal tissue but can exacerbate pathology when activated by injury or disease. Injecting sub-lethal doses of lipopolysaccharide (LPS), activates microglia but without causing damage. This "preconditioning" can be neuroprotective in the brain; however, it is not known if spinal cord microglia also can be preconditioned with LPS.

Hypothesis:

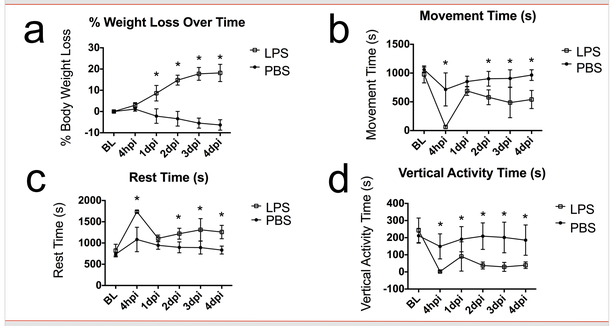


Methods

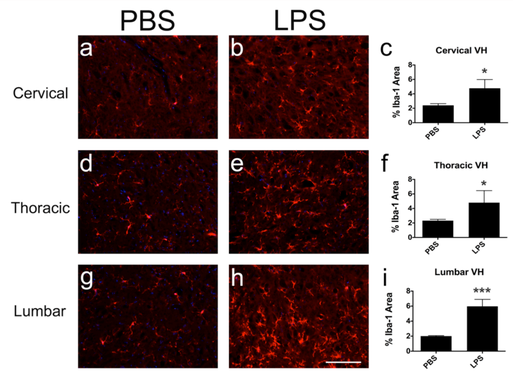


Tissue preparation: Animals perfused with 0.1M phosphate buffer saline (PBS) and 4% paraformaldehyde
Immunohistochemistry: Primary antibodies used: rabbit anti-Iba-1 (1:1000), rat anti-CD16/32 (1:800), rat anti-CD11b (1:1000), rabbit anti-Gal3 (1:4000), mouse anti-iNOS (1:1000), rabbit anti-caspase-3 (1:20,000), rat anti-PECAM1(1:5000).
Microscopy: Iba-1 density, anatomical analysis, and cresyl violet analysis visualize using light microscopy. All other stains visualized using epifluorescent microscopy.
Analysis: Quantification was performed using batch analysis in MetaMorph.

1 LPS injection paradigm induces sickness behavior in mice

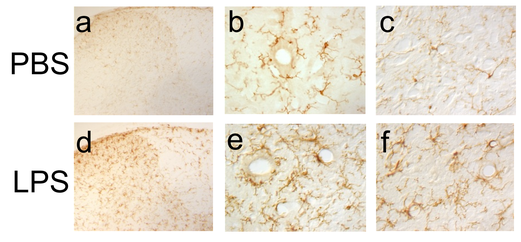


2 Systemic LPS activates spinal cord microglia



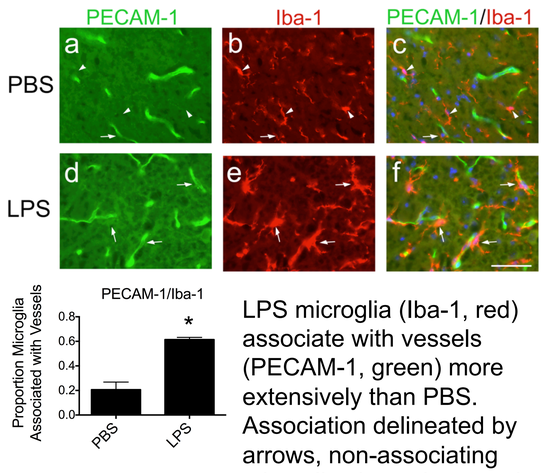
Microglia activation marker Iba-1 was increased in LPS treated spinal cords

3 Primed microglia interact uniquely with spinal cord anatomy



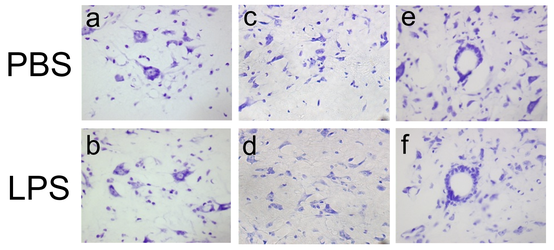
Iba-1 staining shows microglia association patterns. (a,d) LPS treated microglia exhibit increased activation at the dorsal horn. (b,e) Activated microglia associate more readily with the central canal ependymal cells. (c,f) Vessels in LPS treated animals often have microglia surrounding them.

4 More microglia are associated with blood vessels after LPS

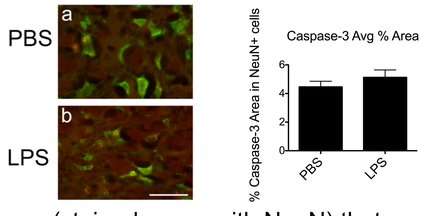


LPS microglia (Iba-1, red) associate with vessels (PECAM-1, green) more extensively than PBS. Association delineated by arrows, non-associating microglia shown by arrowheads

5 Activated microglia do not induce neuropathology

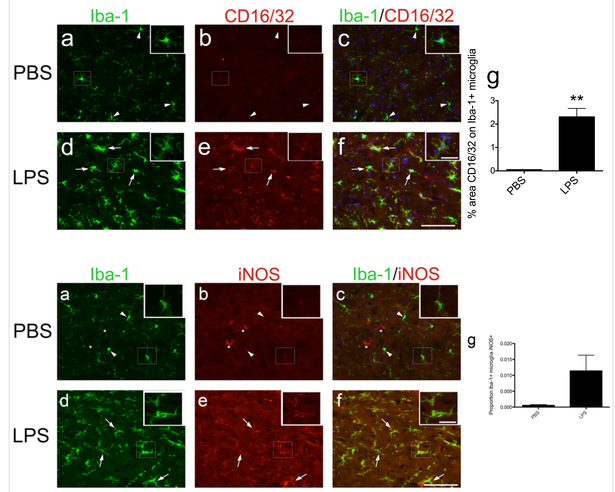


(a,b) Cresyl violet staining indicates normal neuron morphology. (c,d) PBS treated animals have fewer extraneuronal cells associating with neurons. (e,f) Ependymal cells were more rounded and numerous in LPS treated cords.



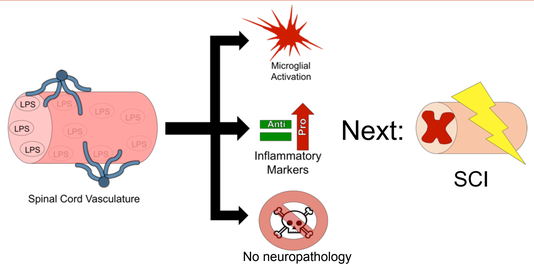
Neurons (stained green with NeuN) that contain apoptotic marker caspase-3 (red). Amount of caspase-3 was not different between groups indicating no changes in neuronal death

6 Primed microglia express elevated levels of inflammatory markers



LPS-treated microglia express higher levels of inflammatory markers CD16/32 and iNOS than PBS controls.

Conclusions



- Peripheral LPS successfully activates spinal cord microglia without inducing neuropathology.
- Future studies will translate this phenomenon to study preconditioning in spinal cord inflammation (i.e. SCI).

References

Ousman SS, Kubes P (2012) Immune surveillance in the central nervous system. *Nature Neuroscience* 15:1096-1101.

Chen Z, Jalabi W, Shpargel KB, Farabaugh KT, Dutta R, Yin X, Kidd GJ, Bergmann CC, Stohlman SA, Trapp BD (2012) Lipopolysaccharide-Induced Microglial Activation and Neuroprotection against Experimental Brain Injury Is Independent of Hematogenous TLR4. *J Neuroscience* 32: 11706-11715.